Evolution of Population Structure in a Highly Social Top Predator, the Killer Whale

A. Rus Hoelzel, Jody Hey,† Marilyn E. Dahlheim,‡ Colin Nicholson,* Vladimir Burkanov,‡ and Nancy Black§*

*School of Biological and Biomedical Sciences, University of Durham, Durham, United Kingdom; †Department of Genetics, Rutgers University; ‡National Marine Mammal Laboratory, National Marine Fisheries Service, Seattle, Washington; and §Monterey Bay Cetacean Project, Pacific Grove, California

Intraspecific resource partitioning and social affiliations both have the potential to structure populations, though it is rarely possible to directly assess the impact of these mechanisms on genetic diversity and population divergence. Here, we address this for killer whales (*Orcinus orca*), which specialize on prey species and hunting strategy and have long-term social affiliations involving both males and females. We used genetic markers to assess the structure and demographic history of regional populations and test the hypothesis that known foraging specializations and matrifocal sociality contributed significantly to the evolution of population structure. We find genetic structure in sympatry between populations of foraging specialists (ecotypes) and evidence for isolation by distance within an ecotype. Fitting of an isolation with migration model suggested ongoing, low-level migration between regional populations (within and between ecotypes) and small effective sizes for extant local populations. The founding of local populations by matrifocal social groups was indicated by the pattern of fixed mtDNA haplotypes in regional populations. Simulations indicate that this occurred within the last 20,000 years (after the last glacial maximum). Our data indicate a key role for social and foraging behavior in the evolution of genetic structure among conspecific populations of the killer whale.

Introduction

Social behavior can affect population structure through an influence on dispersal. The greatest impact will be when both sexes tend to be philopatric. Various factors can promote social behavior and social group philopatry, including antipredator strategies and the cooperative care of young, but here, we focus on foraging behavior. For example, foraging habitat (greater aridity) is thought to promote sociality in the common mole rat (*Cryptomys hottentotus*) (Spinks et al. 2000) and social hunting to reduce energetic costs in African hunting dogs (*Lycaon pictus*; Creel and Creel 1995). Sex-biased dispersal from the natal group is typical, often involving male dispersal in mammalian species (Greenwood 1980). However, resource exploitation could promote philopatry in both sexes if food resources are rare (as for the common mole rat; Spinks et al. 2000) or their efficient exploitation requires significant cooperation, learning, or training. In the latter case, individuals may risk a reduction in fitness when they leave the natal group to join a group that forages on a different resource or by another strategy. This advantage for philopatry would conflict with pressure to outcross. However, to avoid inbreeding depression, we suggest that a strategy of individual social philopatry and "gamete dispersal" could evolve. That is, the individual may remain in the natal social group but reproduce elsewhere during temporary interactions or excursions (this would imply male-biased genetic dispersal in a mammal).

The extent to which the coherence of social groups are influenced by foraging strategy may be determined by various factors including the seasonal distribution of prey, the size and density of prey patches, and the behavior of prey species. However, cooperative interactions among killer whales preying on fish (Hoelzel 1993; Ford and Ellis 2006) and marine mammals (Hoelzel 1991; Pitman et al. 2001) have been described, as have apparent training interactions between parent and offspring (Hoelzel 1991; Guinet and Bouvier 1995).

The killer whale is a highly social resource specialist. Long-term photoidentification studies have provided detailed data on association and movement patterns and little evidence for recruitment to "resident" populations other than by birth or loss other than through mortality (Bigg et al. 1990; Ford et al. 1994; Dahlheim et al. 1997; Matkin et al. 1999). Resident populations were defined in part by foraging specialization on fish prey in contrast to "transient" populations that appear to specialize on marine mammal (primarily) and avian prey (Bigg et al. 1990; Ford et al. 1998; Ford and Ellis 1999). The transient populations were thought to move over a broader geographic range (though this has not been rigorously tested) and have less cohesive social groups (Dahlheim et al. 1997; Ford and Ellis 1999). Sighting data has defined resident populations in different core geographic regions (with ranging behavior that may overlap in some seasons), each composed of multiple pods, none of which have been observed in association with pods from other geographic populations. These data are best for the "southern residents" found seasonally off Washington State and British Columbia, the "northern residents" found off British Columbia (Bigg et al. 1990; Ford et al. 1994), the "Southeast Alaskan residents" (Dahlheim et al. 1997), and the residents found in Prince William Sound (Matkin et al. 1999). More recent studies have begun to document resident populations off the Kamchatka Peninsula, the Aleutians, and in the "Bering Sea", but these are at present less clearly defined. A further putative population typically distributed further offshore (the "offshores"; Ford et al. 1994) is known in less detail. The names of these ecotypes may not fully reflect their behavior or ecology, but they are well established in the literature and will therefore be used here.

Killer whales have revealed very little mtDNA variation worldwide; however, there are fixed differences

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E-mail: a.r.hoelzel@dur.ac.uk.

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between some populations (Hoelzel et al. 1998, 2002). Whitehead (1998) suggested that low mtDNA diversity in matrilineal, social cetacean species (such as sperm whales, *Physeter catodon*, and killer whales) may be explained by "cultural hitchhiking," though several authors expressed reservations about this idea (Mesnick et al. 1999; Schlotterer 1999; Tiedemann and Milincovitch 1999). Hoelzel et al. (2002) suggested that the killer whale had been through a population bottleneck (based especially on tests for neutrality, coalescent based evidence for expansion, and the pattern of diversity) and noted that if so the magnitude of mtDNA differences among populations may sometimes reflect differences among remnant lineages that survived the bottleneck, rather than differentiation related to time in isolation. This together with the matrilineal structure of local populations means that mtDNA is relatively uninformative about migration among populations of this species (except to show that female migration has not occurred between those populations that show fixed mtDNA differences).

Here, we analyze 16 microsatellite DNA loci together with complete mtDNA control region sequences to investigate the pattern of population structure and gene flow (both male and female mediated) among putative populations defined both by geographic range and ecotype. We test predictions about population genetic structure based on the likely consequences of matrifocal sociality and habitat dependence expressed through resource specializations shared by individuals within social groups. Foraging specializations have previously been suggested to influence population genetic structure in another social top predator, the gray wolf (*Canis lupus*; Carmichael et al. 2001). If killer whale social groups found populations when they begin to exploit a local resource, then extant coastal populations in the North Pacific should have been founded after habitat was released from under ice following the last glacial maximum (LGM). If sociality and training are important toward the efficient exploitation of prey, then natal philopatry should be common for individuals of both sexes (as seen in long-term observational studies; e.g., Bigg et al. 1990), and genetic dispersal male biased. If resource specialization leads to differential temporal and spatial habitat use and gene flow takes place during temporary interactions, then the opportunity for those interactions should be relatively rare, especially among groups that pursue very different prey types (e.g., fish vs. mammals), even when their ranges overlap. We investigate each of these predictions.

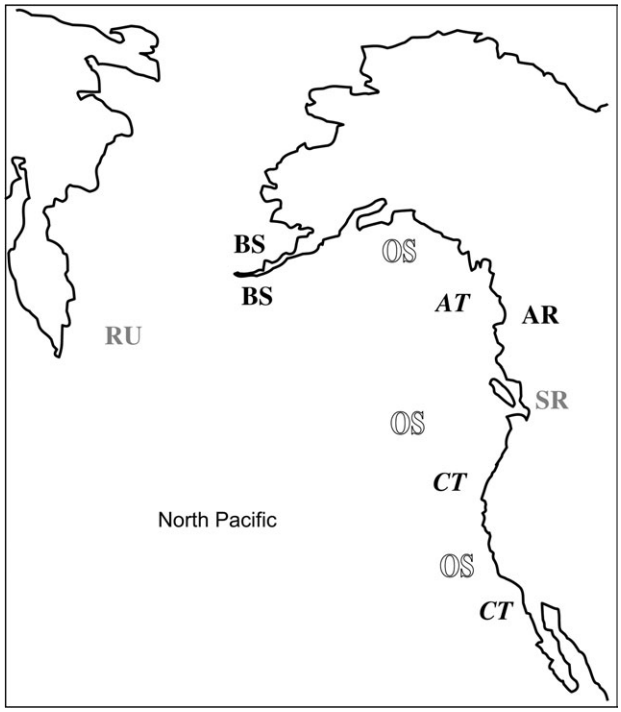

FIG. 1.—Locations of samples and distribution of mtDNA haplotypes: gray bold text = ENPSR; black bold text = ENPAR; italic bold text = ENPT1; outline text = ENPO; see Hoelzel et al. (2002) for sequence data.

Materials and Methods

Sample Collection

Samples were collected from stranded, captive, and free-range whales (the latter by biopsy sampling, see Hoelzel et al. 1998). Regions from which samples were collected are shown in figure 1. The total sample size was 203: 27 from Washington State southern residents (SR), 36 from Southeast Alaskan residents (AR), 14 from a putative resident population off Kamchatka in Russia (RU), 20 from a putative resident population off the Aleutians and in the Bering Sea (BS), 14 from the offshore population (OS), 31 from transient pods sampled in Southeast Alaska (AT), 22 from transient pods sampled in California (CT), and 40 samples collected from the southeast region of Iceland (IC). These sample sizes follow the exclusion of known first order relatives. Iceland was included as an outgroup to provide a sample that would be geographically distant from all North Pacific samples. Populations designated resident are thought to prey primarily on fish, though the data for this is strongest for the southern and Alaskan resident populations. The transient populations are known to prey on marine mammals (see Ford and Ellis 1999). The prey of the offshores is unknown, though stable isotopes and organochlorine analysis suggest possible elasmobranch prey (Herman et al. 2005). The Icelandic whales from the sampled population are known to prey on herring, though the feeding behavior of the sampled pods is not known. DNA was extracted by standard phenol–chloroform methods.

Polymerase Chain Reaction Amplification

Primers for mtDNA situated in $tRNA_{thr}$ and $tRNA_{phe}$ amplify the $tRNA_{pro}$ and entire control region loci for a combined total of 995 bp (for primers and reaction conditions see reference 33; 102 of 203 sequences are from Hoelzel et al. 2002). No variation was found in the $tRNA_{pro}$ region, and therefore this amplification product is referred to as "control region." Amplified DNA was purified on QIAGEN spin columns and sequenced forward and reverse using the ABI 377 automated system. Microsatellite DNA

was amplified from 16 loci. Loci, references, and polymerase chain reaction protocols are provided in supplementary appendix 1 (Supplementary Material online). Amplified microsatellite DNA was analyzed for length variation on polyacrylamide denaturing gels using fluorescent imaging on an automated ABI PRISM 377 DNA sequencer after incorporation of 1/10 fluorescent labeled primer. An internal standard marker (Genescan-500 ROX, Applied Biosystems, Foster City, CA) was used to determine the allele sizes.

Data Analysis

F_{ST} (using the formulations described by Weir and Cockerham (1984) and the significance of its difference from zero were calculated using FSTAT (Goudet 2001). FSTAT was also used to assess evidence for sex-biased dispersal (using sex-specific diversity estimates and assignment indices after Goudet [2001]). This was assessed for just the 3 largest population samples from the North Pacific (SR, AR, and AT) to avoid problems associated with small sample size (significance tested based on 1,000 randomizations). A Mantel test (1,000 permutations) for the correlation between genetic and geographic distance ($F_{ST}/(1-F_{ST})$ vs. ln geographic distance) was run for a subset of the populations using a Spearman's rank correlation test as implemented in GENEPOP 3.1d (Raymond and Rousset 1995a, 1995b). Geographic distances were approximate as the precise delineation of these population ranges is unknown.

The level of genetic diversity was estimated as observed heterozygosity (H_o) and expected heterozygosity (H_e). Evaluation of possible deviations from Hardy–Weinberg was performed using Fisher's exact test and the Markov chain method (dememorization number, number of batches, iteration per batch set at 1,000) using ARLEQUIN (Schneider et al. 2000). Linkage disequilibrium was tested using Fisher's exact test and the Markov chain method (dememorization number, number of batches, iteration per batch set at 1,000), implemented in FSTAT.

The most probable number of putative populations (K) that best explains the pattern of genetic variability was estimated using the program STRUCTURE 1.0 (Pritchard et al. 2000). We assumed the admixture model and performed the analysis considering both the independent and the correlated allele frequency model. Burn-in length and length of simulation were set at 500,000 and 1,000,000 repetitions, respectively. To test the convergence of the priors and the appropriateness of the chosen burn-in length and simulation length, we ran a series of independent runs (4 repeats) for each value of K (for $1 \leq K \leq 9$). We tested whether any particular individual was an immigrant or had an immigrant ancestor by using the model with prior population information, subdividing the individuals into K populations, according to the results of the previous analysis. We assumed v (migration rate) $= 0.05$ and 0.01 (and present the data for $v = 0.05$ because both identified the same set of putative migrants).

An asymmetric estimate of the migration rate ($M = 4N_em$) between a subset of pairwise populations, based on microsatellite data, was calculated using MIGRATE (Beerli 2002). The stepwise mutation model (SMM) was used and long chains were combined for estimates. The lengths of the runs were optimized (acceptance–rejection $> 2\%$, $R < 1.2$). Initial runs were set estimating θ ($4N_e\mu$, where μ is the mutation rate) and M with F_{ST} and allowing M to be asymmetric. Reruns were set using the parameter estimate found with the first run and lengthening the Markov Chain Monte Carlo simulated that is implemented in the MIGRATE program. For comparison, the migration rate was also calculated based on private alleles using GENEPOP 3.1d. This method provides a multilocus estimate of the effective number of migrants (Nm) according to Slatkin (1995). A corrected estimate is given using the values from the closest regression line (Barton and Slatkin 1986).

The data for selected pairs of populations were also considered within an isolation with migration model that explicitly incorporates parameters for time of population splitting, bidirectional gene flow after splitting, and population sizes, including the size of the ancestral population (Nielsen and Wakeley 2001; Hey and Nielsen 2004). The model was fit using a Bayesian framework that provides estimates for the posterior probability density of the model parameters, given the data (using the IM computer program and assuming a SMM; Hey and Nielsen 2004). We used a uniform (i.e., uninformative) prior distribution. This means that in many respects, the parameter estimates are equivalent to maximum likelihood estimates (Nielsen and Wakeley 2001). We ran a number of linked simulations with varying levels of heating (35–80 chains, depending on the populations analyzed) required in order to achieve adequate mixing (Hey and Nielsen 2004). To obtain estimates of effective population sizes (N_e), migration rates, and the time of splitting, we included estimates of the mtDNA control region mutation rate (and scale rates for other loci based on the mtDNA data). With an estimate of the mutation rate, it is possible to obtain estimate of N_1, N_2, N_A (the effective size of populations 1 and 2 and the ancestral population, respectively) and t (time since isolation). Also using estimates of $4N_1\mu$ and m_1/μ (where μ is the neutral mutation rate and m_1 is the probability of migration per generation per gene copy), it is possible to obtain estimates of the effective number of migrants per generation into population 1 (i.e., $2N_1m_1 = 4N_1\mu/(2\ m_1/\mu)$), and similarly for $2N_2m_2$. To accommodate the uncertainty of substitution rates, we used 2 published rates: 1.5×10^{-8} per base pair per year (Hoelzel et al. 1991; Baker et al. 1993) and 7.0×10^{-8} per base per year (Harlin et al. 2003). All of the data sets used for the IM analyses included DNA sequence from the mitochondrial control region, together with 15 microsatellite loci (the FCB4 locus was omitted from these analyses due to the occurrence of large allelic size differences that may violate the assumptions of the SMM). Pairwise comparisons were chosen to be representative and to address key questions about gene flow among and within ecotypes.

Results

There were 6 mtDNA control region haplotypes among the 8 populations included in this study, and each have been reported previously (ENPSR, ENPAR, ENPO, and ENPT1 in the North Pacific and ENAI1 and ENAI2

Table 1
Expected (*He*) and Observed (*Ho*) Heterozygosity Estimates per Microsatellite DNA Locus and Population

	SR		RU		AR		BS		OS		AT		CT		IC	
Locus	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*
MK5	0.807	0.707	0.643	0.696	0.571	0.647	0.800	0.697	0.615	0.615	0.774	0.757	0.762	0.759	0.658	0.640
FCB12	0.435	0.618	0.571	0.611	0.363	0.568	0.450	0.432	0.250	0.496	0.400	0.493	0.650	0.631	0.029*	0.165
EV1	0.455	0.558	0.786	0.688	0.206	0.269	0.550	0.579	0.833	0.721	0.714	0.732	0.682	0.674	0.513	0.531
EV5	0.72	0.630	0.429	0.521	0.389	0.477	0.350	0.388	0.154*	0.526	0.800	0.689	0.591	0.689	0.692	0.753
EV37	0.391	0.619	0.429	0.585	0.677	0.735	0.400	0.599	0.364	0.532	0.500	0.592	0.650	0.790	0.637	0.669
FCB4	0.455	0.555	0.643	0.648	0.833	0.820	0.650	0.869	0.539*	0.837	0.767	0.856	0.947	0.935	0.658	0.734
KW12a	0.926	0.792	0.714	0.608	0.528	0.621	0.684	0.632	0.385	0.705	0.710	0.777	0.857	0.761	0.825	0.692
KW2a	0.583	0.73	0.462	0.646	0.667	0.534	0.235	0.415	0.765	0.779	0.774	0.820	0.809	0.853	0.711	0.686
FCB5	0.727	0.704	0.214	0.262	0.688	0.701	0.500	0.665	0.750	0.851	0.793	0.758	0.667	0.764	0.632	0.732
FCB17	0.583	0.499	0.231	0.517	0.657	0.560	0.461	0.602	0.546	0.437	0.700	0.505	0.571	0.629	0.487	0.529
BA417	0.37	0.307	0.385	0.403	0.177	0.216	0.600	0.510	0.375	0.385	0.484	0.597	0.333	0.340	0.282	0.324
GATA053	0	0	0	0	0	0	0	0	0.077	0.262	0.387	0.428	0.238	0.267	0.410	0.435
GT48	0.316	0.501	0.615	0.649	0.645	0.646	0.647	0.619	0.500	0.579	0.667*	0.844	0.476	0.736	0.559*	0.760
GT142	0.63	0.586	0.500	0.521	0.472	0.520	0.700	0.568	0.231	0.351	0.567	0.516	0.809	0.697	0.825	0.619
MK9	0.579	0.643	0.357	0.537	0.529	0.641	0.421	0.563	1.000	0.811	0.741	0.762	0.833	0.767	0.784	0.670
GATA098	0.148	0.242	0.286	0.508	0.086*	0.311	0.250	0.232	0.231	0.289	0.613	0.552	0.450	0.582	0.316	0.347
Average Gene Diversity	0.420 ± 0.255		0.482 ± 0.264		0.479 ± 0.266		0.506 ± 0.269		0.489 ± 0.278		0.605 ± 0.321		0.591 ± 0.316		0.548 ± 0.292	

NOTE.—SR = southern resident, RU = Russian residents, AR = Southeast Alaskan resident, BS = Bering Sea residents, OS = offshore, AT = Southeast Alaskan transients, CT = Californian transients, IC = Iceland.
* Significant deviation after Bonferoni.

off Iceland; Hoelzel et al. 2002). Within all sampled putative populations in the North Pacific (included in this study), all individuals shared the same population-specific haplotype. The geographically demarcated transient populations in California and Southeast Alaska shared the same single haplotype (ENPT1), whereas all resident-type populations had 1 of 2 haplotypes (ENPSR or ENPAR), and the offshore sample had a separate haplotype (ENPO; fig. 1). The genetic relationship between these haplotypes and the 2 found off IC (uncorrected difference ranging from 0.1% between ENPSR and ENPAR to 0.9% between ENPT1 and ENAI1) has been reported previously (Hoelzel et al. 1998, 2002). A further 4 haplotypes were found among 7 transient whales sampled from Prince Williams Sound to the Aleutians, but this putative population was not well enough sampled to include in this study (data not shown). F_{ST} values were not calculated for mtDNA because variation within most focal populations was zero.

For the microsatellite DNA data, there was neither consistent deviation after Bonferoni correction from Hardy–Weinberg expectations (table 1) nor linkage disequilibrium among locus pairs (significant linkage disequilibrium found only for K2a and MK9 in IC, and MK5 and FCB4 in the Russian population). Measures of F_{ST} comparing the 8 putative populations are shown in table 2. The significance of the difference from zero for each value is given (without Bonferoni correction; the corrected critical value would be 0.0018). The relationship between genetic and geographic distance for the 4 putative resident populations showed a positive correlation (southern residents, Southeast Alaskan residents, resident haplotype whales from the Aleutians/Bering Sea region, and resident haplotype whales from the Kamchatka Peninsula region in Russia; fig. 2; Spearman's rank correlation, $P = 0.04$). Evidence for male-biased dispersal was seen in higher diversity estimates for males within the SR, AR, and AT populations (for males Hs = 0.583; females Hs = 0.547; $P = 0.029$) and weaker assignments (males: mean AI = −0.960; females: mean AI = 0.681; $P = 0.03$).

The assessment of population structure based on Bayesian likelihood estimates (using STRUCTURE) is shown in figure 3. The highest likelihood was found for $K = 7$ populations, and the likelihood value was consistent among the 4 replicate runs. All putative populations were supported with the exception of the Bering Sea population, which appeared to be composed of a mixture of animals from Kamchatka and Southeast Alaska, perhaps indicating a population boundary in that region. Note that all of these animals have the Alaskan resident mtDNA haplotype, as do the Southeast Alaskan residents, whereas the Kamchatka animals have the southern resident mtDNA haplotype. After assigning 7 populations as indicated by the initial runs, we could identify several putative migrants or individuals with migrant ancestry. These are indicated in figure 3 with arrows (omitting the RU and BS populations). Only those with a significance of $P < 0.001$ are shown. One of these suggests a migration event between the offshore and transient communities (between the 2 main mtDNA lineages in the North Pacific; cf., Hoelzel et al. 2002). Also suggested are 2 migrants from RU to AR and migrant ancestry between the transients and the Icelandic population.

Table 2
F_{ST} Values for Pairwise Population Comparisons (lower diagonal), Estimates of Gene Flow Based on the Private Allele Method (upper diagonal), and Directional Estimates from MIGRATE in Parentheses for the North Pacific Samples (left to above followed by above to left)

	SR	RU	AR	BS	OS	AT	CT	IC
SR	—	1.43 (0.48, 0.21)	2.57 (1.10, 0.29)	1.64 (0.50, 0.20)	0.92 (0.55, 0.22)	1.24 (1.44, 0.29)	1.04 (0.62, 0.22)	1.06
RU	0.121**	—	1.90	1.91 (0.99, 0.94)	0.70 (0.85, 0.98)	0.82 (0.88, 1.04)	0.57 (0.99, 0.89)	0.45
AR	0.067***	0.091***	—	3.73 (0.92, 1.10)	1.67 (0.93, 1.08)	1.37 (2.83, 2.18)	0.78 (0.95, 1.07)	0.71
BS	0.077**	0.042**	0.024**	—	0.82 (1.03, 0.97)	1.41 (0.90, 0.97)	0.89 (1.03, 0.93)	0.62
OS	0.162*	0.232*	0.216**	0.211	—	1.68 (0.88, 1.04)	1.51 (1.07, 0.98)	0.62
AT	0.137***	0.169***	0.171***	0.154**	0.102*	—	3.19 (1.04, 0.86)	0.96
CT	0.141***	0.189***	0.171***	0.167**	0.134*	0.037***	—	1.08
IC	0.145***	0.235***	0.193***	0.195**	0.166**	0.145***	0.133***	—

NOTE.—SR = southern resident, RU = Russian residents, AR = Southeast Alaskan resident, BS = Bering Sea residents, OS = offshore, AT = Southeast Alaskan transients, CT = Californian transients, IC = Iceland. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

All pairwise comparisons for estimates of gene flow based on private alleles are shown in the upper diagonal of the matrix in table 2. North Pacific comparisons were also assessed using MIGRATE. Both the maximum likelihood method in MIGRATE and the private allele method assume that populations are in migration/drift equilibrium. In order to assess the role of gene flow in a nonequilibrium model, in which sampled populations may have recently separated from an ancestral population, we fit the data to an isolation with migration model. Parameter estimates from this model, as implemented in the IM program, are provided in table 3, including tests comparing the RU with BS populations reassigned as indicated by the analysis in STRUCTURE. A simple test is used to see if a migration rate estimate of zero can be rejected (Nielsen and Wakeley 2001). Figure 4 illustrates the probability profiles for 2 key comparisons. Using IM, we were able to obtain repeatable posterior density estimates for all included comparisons with one exception (the AR, BS pairing). Overall, there appears to be a strong signal of gene flow in these data, with nearly all compared pairs of populations indicating gene flow in at least one direction. Gene flow rates vary; however, many are greater than or equal to 1, sufficient to prevent the accumulation of appreciable divergence (Wright 1931). The migration results seem to indicate gene flow between even distant populations. In particular, the Iceland and the Alaskan Resident populations show significant evidence of gene flow (from IC to AR), although in this case, the estimated value is the lowest among the statistically significant migration rates found in the study. In principle, the signal could be due to direct gene flow, but more probably to gene flow through other populations.

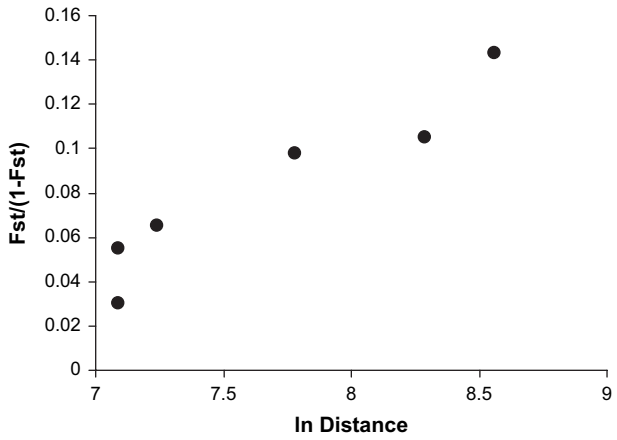

FIG. 2.—Isolation by distance test for correlation between geographic and genetic distance among the 4 putative resident-type populations sampled in the North Pacific. Distance was measured in kilometer.

The locations of the peaks for the N_e and t parameter estimates are shown in table 3, for each of the 2 mutation rates used. A few patterns are evident. First, the effective sizes of regional populations are fairly small, generally under 1,000 and often less than 50 if we adopt the higher mutation rate (table 3). Further, the marine-mammal–eating ecotype showed consistently larger effective population sizes than the fish-eating ecotype (e.g., using the higher mutation rate, the average for AR was 67, whereas the average for AT was 205). Time since divergence was also longer on average for comparisons between ecotypes (using the higher mutation rate, 5,160 compared with 1,714). The regional populations appear to have persisted for thousands of years and possibly, depending on the mutation rate, for tens of thousands of years. A further prominent pattern is that the effective population size estimates of the sampled populations are invariably a small fraction of the estimate for the ancestral population.

Because most analyses yielded findings of significant gene flow, even among geographically separated populations, we were concerned that these estimates might be an artifact of the mutation model used. Specifically, when mutation is biased toward a particular focal length for the microsatellite alleles (i.e., for some allele length x, mutation rates away from x are lower than are mutation rates toward x), this could generate a false appearance of gene flow (Zhivotovsky et al. 1997). Evidence of this kind of bias for microsatellite loci has recently been found in humans and chimpanzees (Sainudiin et al. 2004), and it has the potential to make separate populations appear more similar than they would under the simple stepwise mutation model (Garza et al. 1995; Xu et al. 2000).

To assess the affect of a bias toward a focal length, a modified computer program was written in which the

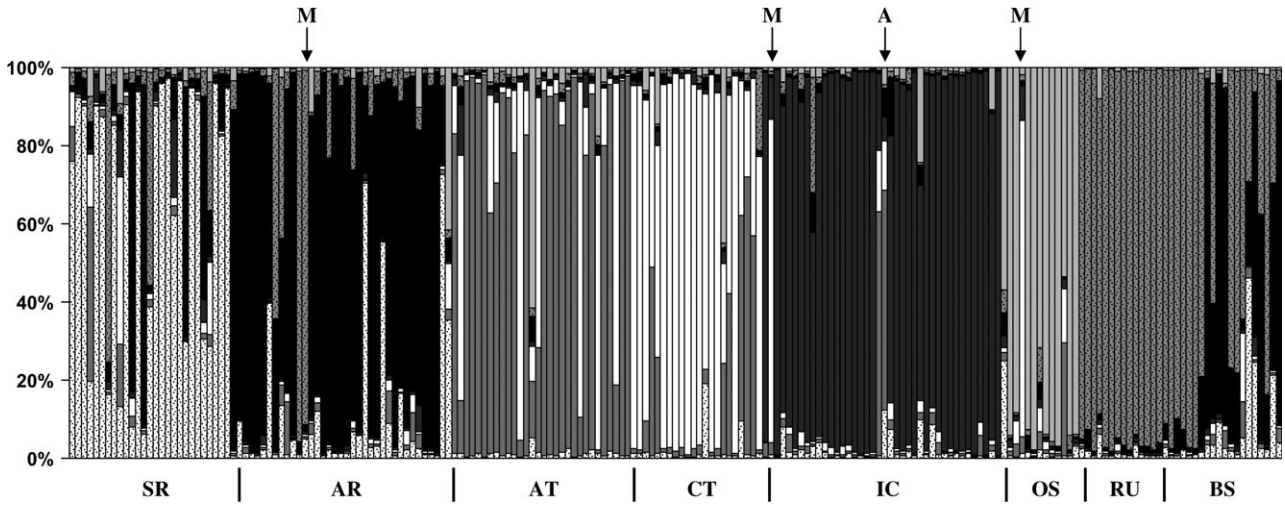

FIG. 3.—Proportional assignment to one of 7 putative populations (in 7 different colors) for each of the 203 individual whales in the study. Population of origin is indicated below the histogram, and putative migrants (*M*) or individuals with migrant ancestry (A) are indicated above with arrows (first arrow identifies 2 significant individuals).

SMM includes a bias toward the most common allele in the sample (for each locus). A single population pair was analyzed (AT, OS) with a mutation bias toward the focal allele length of 0.1. In other words, the mutation rate is set to be higher toward the common allele than it is away from the common allele, by a factor of 10%. The results, listed in the last row of table 3, were very similar to the case with no bias, including the migration results. This suggests that the widespread signal of gene flow is probably not due to a mutation process that is biased toward a common focal allele length.

Discussion

The fact that the local populations of resident pods we studied in coastal habitat throughout the North Pacific are composed of all individuals fixed for the same population-specific mtDNA haplotype suggests that these represent

Table 3
Results from IM Analyses Based on $\mu = 1.5 \times 10^{-8}$ or ($\mu = 7 \times 10^{-8}$), Including Modified (*m*) Groupings Based on Results from STRUCTURE, and a Test Incorporating Mutation Bias (last row)

Population1[a]	Population2[a]	$2N_1m_1$[b]	$2N_2m_2$[b]	N_1[c]	N_2[c]	N_A[c]	T[d]
SR	AT	0.8*	0.6	280 (60)	1,170 (250)	8,000 (1,700)	36,400 (7,800)
SR	AR	0	1*	150 (30)	130 (30)	5,900 (1,300)	7,000 (1,500)
SR	CT	0.6*	0.9	260 (60)	860 (190)	8,200 (1,700)	18,100 (3,900)
SR	OS	0.6	0.7*	180 (40)	310 (70)	6,900 (1,500)	8,100 (1,700)
SR	RU	6.2	2*	150 (30)	10 (3)	4,500 (1,000)	4,300 (900)
AT	AR	0.4	0.8*	1,130 (240)	210 (50)	9,300 (2,000)	21,500 (4,600)
AT	CT	4.4	4.5	520 (110)	310 (70)	4,600 (1,000)	11,400 (2,400)
AT	OS	1.8	1*	1,010 (220)	370 (80)	8,700 (1,900)	26,300 (5,600)
AR	RU	16.9*	1.9*	460 (100)	40 (10)	9,600 (2,100)	6,600 (1,400)
AR	BS	25.1*	2.7	460 (100)	100 (20)	8,700 (1,900)	10,300 (2,200)
CT	OS	0.8	0.8*	860 (180)	310 (70)	8,800 (1,900)	18,200 (3,900)
RU	BS	3.6*	8.7	70 (10)	210 (50)	8,400 (1,800)	8,700 (1,900)
AR	IC	0.2*	0.3	90 (20)	190 (40)	5,700 (1,200)	2,800 (600)
RUm	BSm	3.7*	12*	70 (10)	250 (50)	9,300 (2,000)	13,600 (2,900)
AR	RUm	24.2*	1.7*	450 (100)	60 (10)	8,600 (1,900)	6,700 (1,400)
AR	BSm	16.8*	2.8*	310 (70)	80 (20)	8,900 (1,900)	9,100 (2,000)
SR	RUm	1.1	0.4	240 (50)	40 (10)	5,100 (1,100)	6,200 (1,300)
AT μ bias	OS μ bias	1.9	0.7*	810 (170)	250 (50)	17,900 (3,800)	21,600 (4,600)

NOTE.—SR = southern resident, RU = Russian residents, AR = Southeast Alaskan resident, BS = Bering Sea residents, OS = offshore, AT = Southeast Alaskan transients, CT = Californian transients, IC = Iceland.

[a] The pair of populations used in the analysis, using population designations given in the text. The modified Russian and Bering Strait samples, based on the STRUCTURE analysis as described in the text, are designated RUm and BSm.

[b] The estimated effective number of migrant gene copies per generation in each direction (into Population1 and into Population2, respectively).

[c] The estimated effective population sizes for Population1, Population2, and the Ancestral population, respectively.

[d] The estimated time in years at which the ancestral population split into the 2 sampled populations.

* Statistically significant at $P < 0.05$. Significance levels were not adjusted for multiple comparisons because separate tests are likely to be independent, due to uncertainty about the phylogenetic relationships among groups and the degree to which gene flow with nonsampled populations could contribute to the appearance of gene flow in any particular comparison.

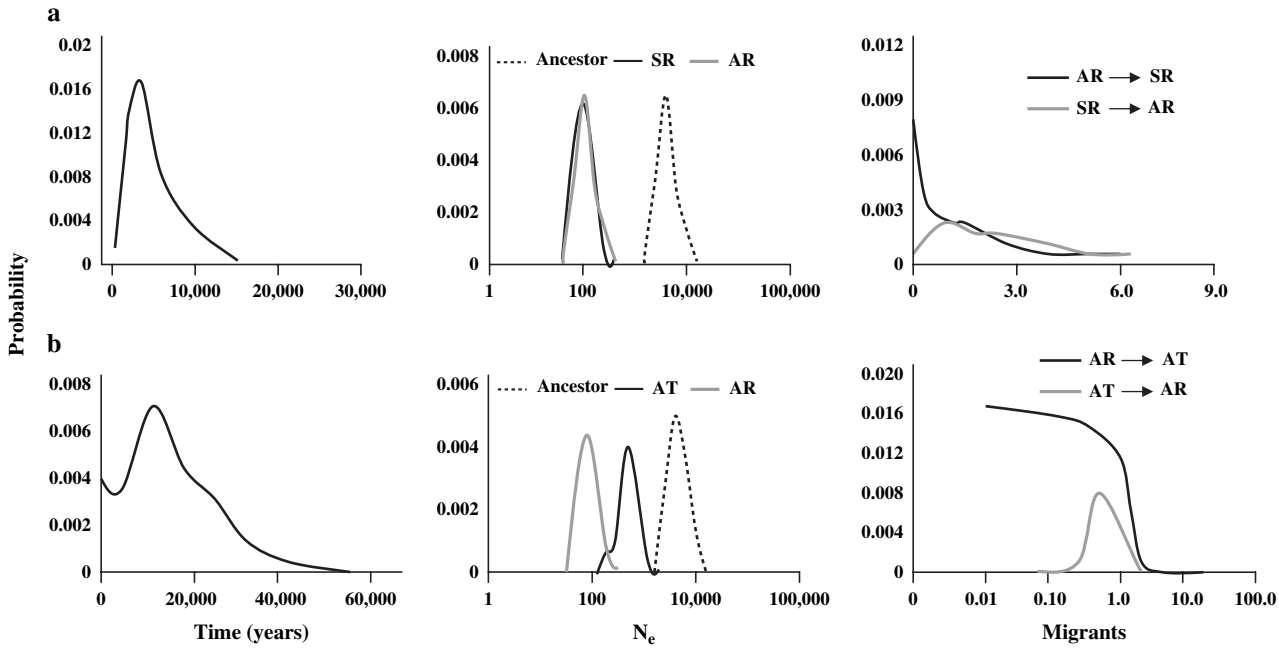

FIG. 4.—Illustrations of the distributions representing estimated time points of divergence, effective population sizes of the extant and ancestral populations, and the effective number of migrants per generation ($2Nm$) for the comparison between (*a*) the Southern and Alaskan resident populations (*b*) and between the Alaskan resident and transient populations. Illustrated results are based on $\mu = 1.5 \times 10^{-8}$.

local founder events, perhaps initially founded by single matrifocal pods. Without further female immigration from other matrifocal groups, these populations could expand, generating new pods by fission, and retain the same mtDNA haplotype among all individuals in the extended clan. Effective population size estimates were relatively low for resident populations in coastal habitat. The 1-bp mtDNA control region difference between the 2 resident haplotypes (see Hoelzel et al. 2002) suggests recent coancestry, and perhaps historical occurrence in a common population prior to the founding of the extant coastal populations. The founding may have occurred after the habitat became available following the last glacial epoch, and the IM divergence time estimates were consistent with this. The division between ecotypes may be older than divisions within ecotypes, though all appear to have occurred since the LGM. The distribution of the same closely related haplotypes among populations across this wide geographic range (fig. 1) indicates that the haplotypes diverged in a common source population prior to the founding of the coastal populations. Although we still know little about behavior in transient pods, they appear to be more fluid in composition and may find resources over a broader geographic range (see review in Baird 2000). These factors could lead to greater outcrossing and consequently larger N_e (as indicated from the IM analyses).

Measures of F_{ST} among putative populations were comparable in magnitude to population differences in other species of large mammals (e.g., Kermode bears (*Ursus americanus kermodei*) in British Columbia (Marshall and Ritland 2002); lynx (*Lynx lynx*) in Scandinavia (Rueness et al. 2003); Wolves in Europe (Lucchini et al. 2004); bottlenose dolphins (*Tursiops truncates*) in the Mediterranean Sea (Natoli et al. 2005)). Most putative populations identified by both geography and ecotype were well supported by the assignment method implemented in STRUCTURE. Clear geographic structuring was seen in spite of the fact that killer whale social groups are known to be capable of excursions of at least several thousand kilometers (Stenersen and Similä 2004). The system from the Kamchatka Peninsula to the BS (fig. 1) will require further resolution, though significant differentiation was found based on F_{ST}, and the alternative IM analysis (based on assignments from STRUCTURE) did not increase evidence for isolation (table 3). Isolation by distance was suggested within an ecotype by the clear pattern in figure 2 (though the small number of populations meant low statistical power). At the same time, different ecotypes showed F_{ST} values in sympatry that were of the same magnitude as among populations of the same ecotype on either side of the Pacific. The implication is that both geographic distance and ecotype specialization are important in structuring populations. The magnitude of gene flow estimates are very similar for comparisons within and between ecotypes (see tables 2 and 3). A comparative analysis of male and female data on population structure supported the hypothesis that genetic dispersal under this model should be mediated by males (though this is common for mammals in any event).

It is possible that the sampled regional populations all evolved as founders from one or a few larger ancestral populations. For example, the coastal habitat occupied by these populations only became available after the ice retreated some 10,000–15,000 years ago. Other species (e.g., bottlenose dolphins) show large, diverse offshore populations and smaller regional coastal populations that are genetically differentiated from each other (Natoli et al. 2004, 2005). However, an important possible alternative explanation for the large ancestral population size from IM estimates is that the ancestors of these populations were themselves

exchanging genes. In that case, a large estimated effective size for the ancestral population is expected as a byproduct of the method, which assumes that there was only one constant sized ancestral population (Won et al. 2005). If the ancestral populations had been exchanging genes, the apparent ancestral effective size would be closer to the actual N_e for the species as a whole. Nonsampled populations are another important consideration as apparent gene flow between a pair of populations could instead represent migration via intermediary populations (as is most likely the case for the comparison between Iceland and the Alaskan residents).

Estimates of effective population size and divergence time are dependent on an estimate of mutation rate that cannot be known precisely (due to uncertainty about palaeontological calibration points and the suitability of alternative substitution models). However, it is possible to assess putative rates in the context of the estimates generated. In this case, the higher rate provides more realistic estimates for both N_e and the implied rate of mutation at the microsatellite DNA loci. Effective population size was found to range from approximately 10–50% of census population size in a meta-analysis (Frankham 1995), so for example, the average N_e for SR of 45 would imply a census size of 90–450, whereas the recent actual population size has been ~100 whales. The higher mtDNA rate (7×10^{-8}) implies an average mutation rate for the microsatellite loci of approximately 2×10^{-4}, consistent with average estimates obtained for other mammalian species (Dallas 1992; Ellegren 1995; Whittaker et al. 2003). Estimates of divergence time were on average more recent than the LGM in the North Pacific based on either mutation rate.

Energy transfer is relatively inefficient for top predators, and therefore, the social facilitation of prey location and capture may have evolved in some species, such as the killer whale, as a strategy to maximize capture rate. If cooperative groups learn information on prey distribution in time and space, and on strategies for their efficient capture, and this differs among groups, then this could affect dispersal patterns and rates. As long as this learning "tradition" remains relevant (in the context of changing environments), it could continue along social matrilines and may contribute to their regional differentiation (reflected in mtDNA diversity; cf., Whitehead 1998). However, these traditions would be different for each local population (and so maintain matriline diversity) and not immutable. A changing environment may force a group to learn new strategies and possibly make new associations. We find evidence for male-mediated dispersal at a rate high enough to maintain some genetic similarity among populations. When this genetic dispersal occurs in temporary associations, there may not be much opportunity for the transfer of learned strategy, but males moving to a new group could do so.

Strict matrifocal sociality for the killer whale is reflected in the fixed mtDNA haplotypes in regional populations. Philopatry, consistent with that indicated from observational studies, is indicated by the large, significant F_{ST} values seen among regional populations, and the relatively low estimates of gene flow. The role of ecotype in the isolation of populations is indicated by the large F_{ST} values among different ecotype populations in sympatry (though the IM model indicates ongoing gene flow). If gene flow is male mediated during temporary associations, as we propose, then differentiation should be related to the probability of interactions, which could be expected to be rarer at greater geographic distance (supported by the data on isolation by distance within the resident ecotype), and between ecotypes given different temporal and fine-scale spatial use of a given area (Hoelzel 1993), again supported by our data. Taken together, our data support the hypothesis that strict sociality in groups that specialize on local prey resources leads to male and female social philopatry (with males mediating gene flow in temporary associations) and a process of population founding and expansion that drives the evolution of population genetic structure in this species.

Supplementary Material

Supplementary Figure is available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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